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Identification of Metabolites of Pentachlorobenzene and 1,2,4,5-Tetrachlorobenzene in Coyote Feces: Development of Physiological Markers for Wildlife Damage Control

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Abstract: Coyotes were dosed with pentachlorobenzene (PeCB) and 1,2,4,5-tetrachlorobenzene (1,2,4,5-TeCB). Residues were determined in feces and adipose tissue at intervals up to six months post-dosing. PeCB was detectable in feces for six months post-dosing. 1,2,4,5-TeCB residues were of similar persistence and magnitude to those of PeCB. PeCB was metabolized to pentachlorophenol and 2,3,4,5-tetrachlorophenol. Simultaneous oral delivery of two biomarkers decreased absorption by about 25%. These data indicate that PeCB and 1,2,4,5-TeCB have excellent applicability as long term biomarkers for wildlife study and management.

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1 INTRODUCTION

In 1991, US agriculture sustained an estimated $41·5 million in losses due to predation on cattle and calves. Coyotes (*Canis latrans* Say) were responsible for the bulk of cattle and calf predation; losses due to coyotes were valued at $24·3 million.[1] In 1990, losses of sheep and lambs to predators were estimated at $21·7 million, with $13·6 million attributed to predation by coyotes.[2] In an effort to minimize losses due to coyote predation, a variety of techniques are currently employed. These techniques include modification of animal husbandry practices, implementation of physical barriers, use of frightening devices, killing and/or relocation.[3] While most of these animal damage-control techniques are rather indiscriminate, preliminary observations indicate that only certain coyotes within a pack are responsible for predation on livestock.[4,5] We believe that the development of physiologic marking agents which can be selectively delivered to those coyotes which attact livestock will allow for research leading to a more thorough understanding of coyote behavior. Such knowledge is essential for the development of selective coyote control techniques. Other potential applications of physiologic marking agents include estimation of animal abundance and the proportion of populations that ingest baits delivering substances such as toxicants, vaccines or contraceptives. They also have potential utility for quantitative studies of social behavior.

Selection of compounds for study as potential physiological biomarkers focused on compounds which would be relatively non-toxic to study animals, easily deliverable and detectable for a required period of time (usually several months). Further criteria include the selection of matrices which can be sampled in a non-intrusive manner yet permit easy quantitation of analytes with minimal sample preparation.

We viewed lipophilic chlorinated benzene compounds as promising candidates, as they have excellent potential for extended persistence and can be quantified *via*

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highly selective gas chromatography/electron capture detection (GC/ECD). During passage through the GI tract following ingestion, hexachlorobenzene was absorbed mainly in the jejunum and ileum by the lymphatic system and subsequently bioaccumulated and stored in adipose tissue.[6] We have previously shown pentachlorobenzene (PeCB) to be a promising biomarker as residues in feces and serum were detectable for up to four months following a 100 mg dose.[7] A 100 mg dose of 1,2,3,4-tetrachlorobenzene (1,2,3,4-TeCB) resulted in detectable residues for only eight days. The metabolite 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP) was detected in feces collected from 1,2,3,4-TeCB-dosed animals. As metabolites may offer detectability for longer duration than the parent compounds, we analyzed feces from PeCB-dosed coyotes for the presence of metabolites, beginning at approximately 2·5 months post-dosing, when the residue levels of the parent compound began to approach the method limit of detection. To determine if the concentration of feces analytes is indicative of the body burden of the parent compound, PeCB adipose residues were determined in tissue collected pre- and 114 days post-dosing. As there is a potential need for the simultaneous use of multiple distinguishable biomarkers, we compared the persistence of 1,2,4,5-tetrachlorobenzene (1,2,4,5-TeCB) with that of PeCB as potential wildlife biomarkers. Feces collected from these coyotes were also screened for metabolites. As oral dosing is the most practical route of application for wildlife studies, animals were orally dosed with chlorinated benzene compounds dissolved in mineral oil.

As feces samples may be of different ages when collected during field studies, we quantified the parent compound and phenolic metabolites in dried feces. In this study, coyote urinary excretion was not determined, as urine is not a practical matrix for wildlife sampling under field conditions. Also, metabolism studies with the Rhesus monkeys and rats showed the major excretory pathway for PeCB to be feces; feces contained twice or three times more PeCB and metabolites than did urine.[8,9] One would expect the urinary residues of PeCB and TeCB to be low.

2 MATERIALS AND METHODS

2.1 Animal procedures

Coyotes used as subjects were captives (2–12 yrs of age) held in covered outdoor kennels (1·2 × 3·6 × 1·8 m) at the USDA Predator Research Facility, located 12 km south of Logan, Utah. Coyotes received a daily ration (approx 300 g) of a commercial dry dog food (Hill's® Pet Nutrition Inc., Topeka, Kans.). For dosing and blood sampling, all coyotes were immobilized with an IM injection of 100 mg ketamine hydrochloride (Aveco Co., Inc., Fort Dodge Ia) and 1 mg acepromazine maleate (Fermenta Animal Health Co., Kansas City Mo). For surgical sampling of adipose tissue, coyotes were anesthetized with sodium thiopental (Abbott Laboratories, North Chicago, Ill) at an average dose of 0·2 mg kg^{-1} administered intravenously for the necessary effect. Coyotes were weighed on a platform scale, and their body length (tip of nose to base of tail) measured with a steel tape. For an index of subcutaneous adipose tissue, skinfold thickness was measured using a Harpenden skinfold calipers (British Indicators Ltd, St. Albans, Herts, UK) in the lumbar region and between the scapulae.[10]

2.2 Formulations and standards

For comparison of residues and metabolites following dosing with PeCB and 1,2,4,5-TeCB, the compounds were formulated in light mineral oil (Aldrich Chemical Co.) at concentrations of 49·5 and 18·6 mg ml^{-1}, respectively.

2,3,4,5-tetrachlorophenol (TeCP) and 2,3,4,6-TeCP standards for chromatographic and mass spectral analyses were obtained from Supelco (Belefonte, PA) and Chem Services (West Chester, PA), respectively. Pentachlorophenol (PeCP) was obtained from Aldrich Chemical Co.

2.3 Dosing procedures

For assessment of the relationship between dose and parent and metabolite residue concentrations, 12 coyotes were randomly assigned to three treatment groups of three animals, along with three untreated controls. Each group had at least one animal of each sex. Oral treatments of 130, 260 or 520 mg PeCB were administered to coyotes pre-fasted $\geq$10 h on 2 May 1995. Individuals receiving 130 mg were dosed by feeding the formulation in gelatin capsules during recovery from immobilization; coyotes in the control and highest treatment groups were dosed *via* a gavage tube while immobilized. The quantities of formulation delivered per treatment were 2·5, 5 and 10 ml, respectively, and controls received 10 ml of mineral oil.

We compared fecal residue concentrations in animals treated simultaneously with PeCB and 1,2,4,5-TeCB versus single doses of each compound. Fourteen coyotes were randomly assigned to three treatment groups of four animals, and two coyotes were untreated controls. Each group was composed of equal numbers of males and females. Oral doses of 99·0 mg PeCB and/or 48·4 mg 1,2,4,5-TeCB were fed to coyotes in gelatin capsules containing 4 ml of formulation on 16 January 1995. The controls received 4 ml of mineral oil.

2.4 Sample collection and preparation

Fresh feces for analysis of the concentrations of PeCB and TeCB were collected from kennels of coyotes during the morning of each sampling date. Fecal samples of 100–300 g included representative portions of the deposits accumulated from each coyote during the preceding 20 h. The composite samples were manually homogenized and dried fecal samples were prepared by placing 15–60 g of fresh feces in a forced-air laboratory oven at 35–40°C for six days. Subsamples were weighed in 50-ml glass tubes and stored at room temperature. Adipose tissue samples were collected at 30–40 days ($\bar{x} = 35$ days) pre-treatment and 111–118 ($\bar{x} = 114$ days) post-treatment. Adipose tissue samples (1·5–2·5 g) were excised from the falciform-ligament deposit *via* surgical procedures. Three subsamples (0·5–1·0 g) were weighed in 50-ml glass tubes, and frozen.

For the comparison of single and combined doses of PeCB and 1,2,4,5-TeCB, feces were collected at seven days pre-treatment and 1, 3, 7, 14 and 28 days post-treatment.

2.5 Quantification of biomarkers

Marker residues were quantified by injecting 1 μl of extract into a Hewlett Packard 5890 gas chromatograph (GC) equipped with a 60 m × 0·31 mm ID (0·25 μm film thickness) J&W (Folsom, CA) DB-5 (5% phenyl-methylpolysiloxane) column operated with an oven program of 90°C for 1 min, 40°C min^{-1} ramp to 195°C, 5 min hold at 195°C, 40°C min^{-1} ramp to 285°C, hold for 5 min. A detector temperature of 325°C and injection port temperature of 295°C were used. Helium was used as a carrier gas at 1 ml min^{-1} and as the make-up gas for the electron capture detector (ECD) at 55 ml min^{-1}.

Owing to the limited linear range of the EC detector, linear regression equations were generated from three standard curves prepared at 3–125 ng ml^{-1}, 125–9500 ng ml^{-1} and 9500–100 000 ng ml^{-1}. Concentration of analytes in sample extracts were determined using the linear regression equation from the standard curve containing the appropriate detector response. Potential matrix interferences were assessed by quantification of the response at the expected analyte retention time in the chromatograms from the analyses of extracts from each control matrix. Using a single point calibration from the analysis of a 3 ng ml^{-1} chlorobenzene solution, the instrument limit of detection was calculated as the concentration of analyte required to generate a response equal to 2 x baseline noise observed at the identical retention time in a chromatogram from the analysis of a reagent blank. The method limit of detection (MLOD) was similarly calculated from the analyses of control matrices fortified at 40 ng ml^{-1} as the concentration of analyte required to generate a response equal to 2 x baseline noise at the identical retention time in a chromatogram from the analysis of a control matrix extract.

Quality control samples were prepared in triplicate for each matrix at concentrations approximating to the expected results for each analytical run. The daily QC mean recoveries were used to normalize the daily residue results.

Storage stability was ascertained by individually homogenizing feces from coyotes which were dosed with 50 mg each of technical 1,2,4,5-TeCB and PeCB. Analyte concentrations were determined after 0, 30 and 60 days of storage at $-27(\pm 2)$°C. The relationship of QC-corrected residue concentrations versus time was analyzed by Linear Regression Analyses using the SAS General Linear Model Procedure. Degradation was indicated by a negative slope which was significantly different from zero and significantly different residue concentrations for each sampling period.

2.6 Metabolite identification

To obtain sufficient quantities of suspected PeCB and TeCB metabolites for spectral identification, feces from coyotes receiving the same dose level and containing metabolites tentatively identified by GC/ECD were combined and extracted. The extracts were concentrated and analyzed on a Hewlett Packard 5890 Series II GC equipped with a HP 5972 Mass Selective Detector. GC conditions were as stated above. Mass spectral detection was conducted in both the total ion scan ($m/z = 100$–300 m/z) and selected ion monitoring ($m/z = 131, 230, 232, 240$) modes. Differentiation of TeCP isomers and secondary column confirmation of PeCP was accomplished by GC/ECD analysis with a 15 m × 0·25 mm (0·25 μm film thickness) FFAP (acidified propylene glycol) column (J&W Scientific) using a temperature program of 100°C for 0·5 min, ramp at 45°C min^{-1} to 215°C, hold for 4 min, ramp at 30°C min^{-1} to 240°C, hold for 18 min. Other GC conditions were as stated above.

To investigate the presence of conjugated metabolites, 1-g subsamples of pooled feces (tentative positive and controls) were treated with 2 mg β-glucuronidase (Sigma) and 4 mg sulfatase H-1 (Sigma) in 3·5 ml 4 mM pH 4 and 5 phosphate buffer, respectively, and incubated at 37°C for 30 min with frequent shaking. Incubates were acidified with 1 drop 1 M hydrochloric acid to enhance the recovery of phenolic metabolites, extracted and analyzed by GC/ECD with the FFAP column. Positive enzyme activity was monitored by identical treatment of *p*-nitrocatechol sulfate- and phenylglucuronide-fortified control feces and subsequent quantitative recovery of *p*-nitrocatechol and phenol. Additional controls included sulfate- and glucuronide-fortified feces which were incubated in the absence of enzyme and subsequently analyzed.

3 RESULTS

3.1 Method limits of detection

GC/ECD chromatograms of extracts of feces and adipose tissue collected from coyotes dosed with PeCB and TeCB are shown in Fig. 1. MLOD varied slightly with the day of analysis. The mean daily MLODs were 50 $\mu g\ kg^{-1}$ PeCB, 30 $\mu g\ kg^{-1}$ 1,2,4,5-TeCB, 70 $\mu g\ kg^{-1}$ 2,3,4,5-TeCP and 250 $\mu g\ kg^{-1}$ PeCP for feces. For the analysis of adipose tissue extracts, the MLODs were 8 mg kg^{-1} PeCB and 12 mg kg^{-1} TeCB. The concentration of the analytes in feces did not change significantly during the storage stability study.

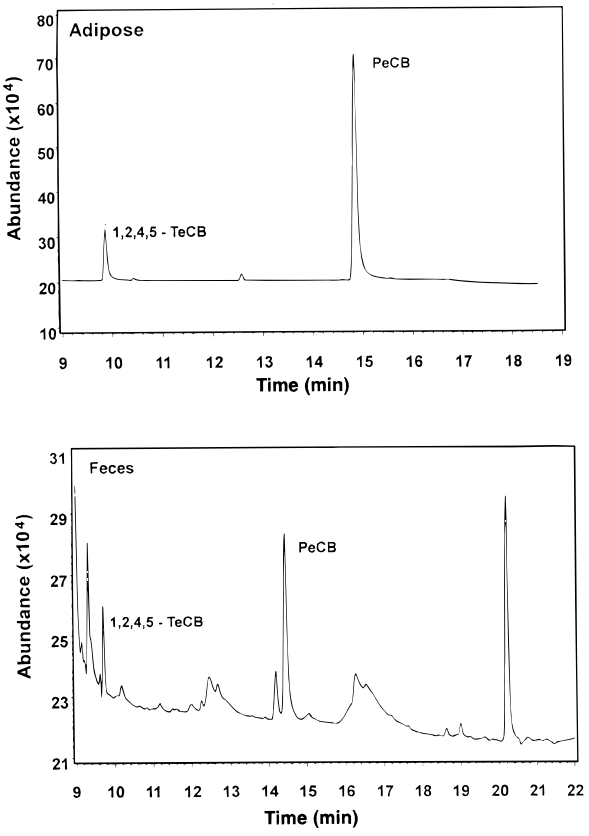

Fig. 1. GC/ECD chromatograms of extracts from adipose tissue and feces collected from coyotes orally dosed with 100 mg PeCB and 50 mg TeCB in oil.

3.2 Dose level versus residue levels

Coyotes received doses ranging from 130–520 mg for the study which investigated the effects of varying dose on residue levels. As indicated in Fig. 2, the higher dose generally resulted in higher feces residue levels. The adipose tissue residue levels presented in Table 1 show a linear relationship between oral dose level and residue levels (corr. coef = 0·97). Higher doses of PeCB resulted in a proportional increase in adipose tissue residues.

Linear regression analyses were conducted to investigate the relationships between residues in feces and

TABLE 1
Mean Residues in Adipose Tissue Sampled at 114 Days Post-Dosing

Dose—Route of delivery	*Biomarker* residues ($\mu g\ kg^{-1}$)
50 mg 1,2,3,4-TeCB Solid oral dose	180
50 mg PeCB Solid oral dose	210
65 mg PeCB IM injection	3200
65 mg PeCB Oral dose	1670
130 mg PeCB Oral dose	2750
260 mg PeCB Oral dose	6580
520 mg PeCB Oral dose	20 190

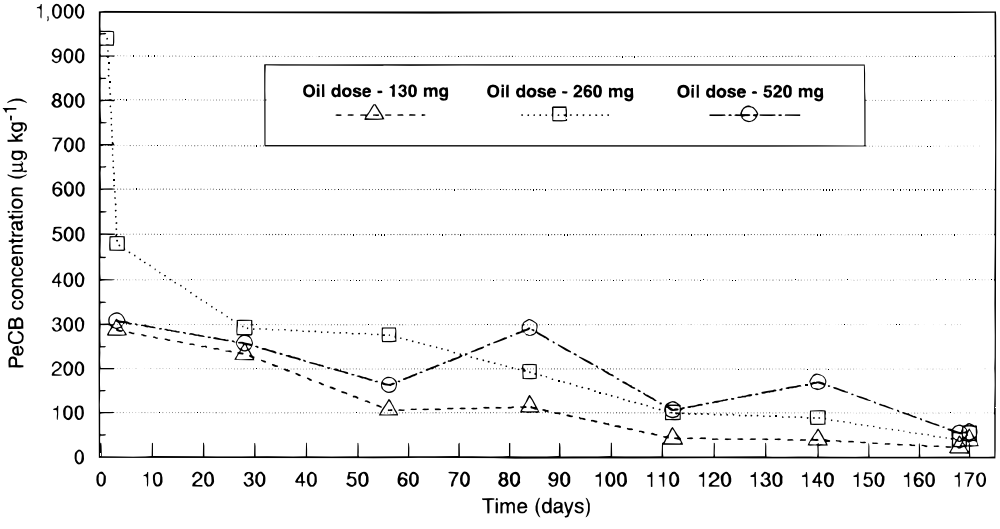

Fig. 2. The effects of varying PeCB oral dose level on residue levels in feces.

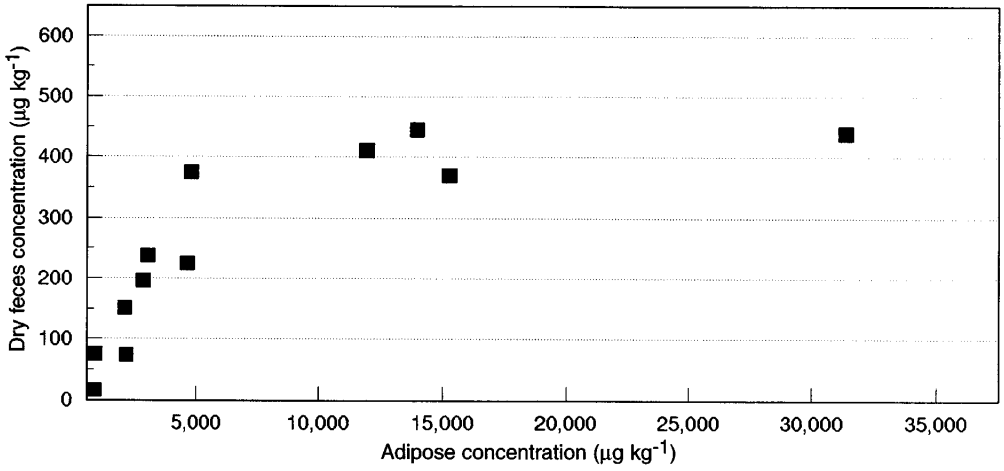

Fig. 3. Relationship of adipose tissue versus feces PeCB levels.

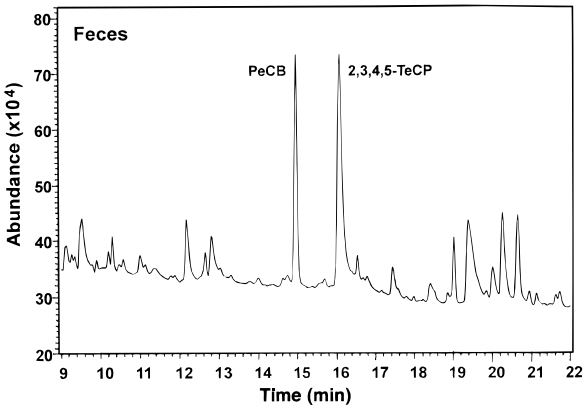

Fig. 4. Chromatogram of feces extract from PeCB-dosed coyote, indicating PeCB and 2,3,4,5-TeCP.

those in adipose tissue (Fig. 3) for residue data from coyotes dosed with 130, 260 and 520 mg PeCB. The relationship appeared not to be linear over the entire range for feces versus adipose tissue PeCB concentrations. However, for fat concentrations less than 300 μg kg^{-1}, the correlation coefficient was 0·88. The relationship for higher concentrations resulted in greater variability and a lesser slope.

3.3 Metabolite identification

A GC/ECD chromatogram of pooled feces from coyotes receiving 520 mg of PeCB is shown in Fig. 4.

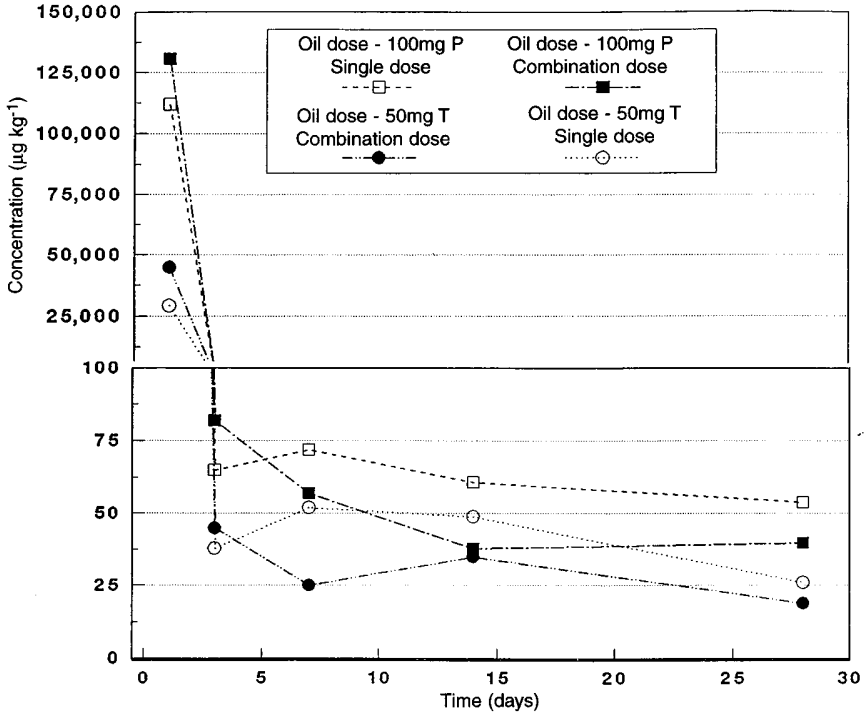

Fig. 5. The effects of single versus combined biomarker dose on residue levels in feces.

The chromatogram indicates the presence of a suspected metabolite at retention time 16·2 min in addition to PeCB at 15·1 min. A broad late-eluting peak (c. 31 min, not shown) was indicative of a possible second metabolite. The early-eluting metabolite (16·2 min) co-chromatographed with the 2,3,4,5-TeCP and 2,3,4,6-TeCP standards while the late-eluting suspected metabolite co-chromatographed with the PeCP standard. The early-eluting metabolite was tentatively identified in feces from the coyotes dosed with 130, 260 and 520 mg PeCB as a tetrachlorophenol (TeCP) based on its mass spectrum: *m/z* (relative intensity): 131(80), 230(81), 232(100), 234(50). It was not possible to distinguish between the TeCP isomers based on the mass spectrum of the unknown. Due to the poor chromatography of the late-eluting metabolite, a quality mass spectrum was not obtainable. Analyses of the feces extract, PeCP and both TeCP standards by GC/ECD with a FFAP column confirmed the identity of the early-eluting metabolite as 2,3,4,5-TeCP. The late-eluting metabolite was confirmed as PeCP. PeCP was also detected in the feces extracts from coyotes dosed with the 130, 260 and 520 mg PeCB. PeCP concentrations were about twice those of 2,3,4,5-TeCP. Enzymatic hydrolysis studies produced no increase in the quantity of 2,3,4,5-TeCP or PeCP detected in feces extracts.

3.4 Single versus combined dose

A comparison of the mean feces residue data for coyotes receiving a combined or single biomarker dose is represented in Fig. 5. As expected, the 100-mg PeCB doses resulted in higher residues than did the 50-mg TeCB dose. However, on day 1, the combined-dose coyotes eliminated feces with higher residue concentrations than did the coyotes receiving a single biomarker dose. By day 3, the feces residues were higher for the coyotes which received the single dose. For all sampling intervals, the feces residues were greater for the coyotes dosed with the single biomarker. Generally, residues at day 28 for the oil-dosed coyotes were at least ten times greater than the MLOD.

4 DISCUSSION

The risk of PeCB and TeCB acute toxicity was considered to be minimal, as the reported oral LD_{50} doses for rats were 1125 and 1470 mg kg^{-1}, respectively.[11,12] and the highest single dose administered to coyotes in this study was approximately 45 mg kg^{-1}. No evidence of acute toxicity was noted in coyotes, based on external symptoms. Similarly, in our previous study,[7] coyotes dosed with PeCB at 100 mg kg^{-1} showed no abnormal external symptoms or serum biochemical changes.

The potential for subacute toxicity from ingestion of chlorinated benzenes poses a greater risk to animals than does acute toxicity.[11–13] The subacute toxicity of PeCB and the three isomers of TeCB was assessed during a 28-day feeding study in rats.[14] No clinical signs of toxicity nor effects on weight gain were observed in rats fed diets of 5, 50 and 500 mg kg^{-1} PeCB and 1,2,4,5-TeCB. These are approximately equivalent to dosages of 0·25, 2·5 and 25 mg kg^{-1} day^{-1}, respectively.

Although our study involved only single doses of PeCB and TeCB, their absorption in adipose tissue and subsequent elimination and metabolism still posed a subacute risk to coyotes. Examinations during post-dosing sampling revealed no visible symptoms of toxic effects in treated coyotes. Mean body mass of coyotes did not differ among treatment groups at dosing ($F_{10,24} = 0{\cdot}2$, $P > 0{\cdot}50$) and was unchanged ($F_{7,16} \leq 0{\cdot}8$, $P > 0{\cdot}50$) among groups from dosing to day 140.

These results indicate that PeCB has excellent potential as a physiological biomarker for long-term wildlife studies, as feces residues were detectable (>MLOD) for at least six months post-dosing. Our previous study[7] indicated that 1,2,3,4-TeCB was not detected in sera and feces beyond eight days post-dosing. 1,2,3,4-TeCB is not a likely candidate for a long-term physiologic biomarker for wildlife.

However, there are wildlife management situations requiring multiple vaccinations at intervals of several weeks for which 1,2,3,4-TeCB may be well suited. Turner *et al.*[15] showed that multiple vaccinations at three-week intervals increased the efficacy of immunocontraceptive vaccines in deer. The distribution of such a vaccine administered orally in wildlife populations could be monitored by administration of PeCB with the initial vaccination and 1,2,3,4-TeCB with subsequent vaccinations. Collection and analyses of feces during the week following the subsequent vaccinations would permit an estimation of the percentage of a wild population receiving the initial and subsequent vaccination.

As field application situations may require the simultaneous use of multiple long-range biomarkers, we evaluated 1,2,4,5-TeCB for this purpose. Owing to the limited solubility of 1,2,4,5-TeCB in the oil carrier, coyotes were dosed with 99·0 mg PeCB and/or 48·4 mg 1,2,4,5-TeCB. PeCB residues were about twice those of TeCB (Fig. 5), suggesting that, for a comparable dose, 1,2,4,5-TeCB and PeCB may have a similar longevity as a biomarker. The resulting residues in feces collected from coyotes administered a combined dose of PeCB and 1,2,4,5-TeCB were about 25% lower than from animals administered either biomarker individually. This minor difference suggests that the simultaneous use of PeCB and 1,2,4,5-TeCB is possible in situations requiring two separate and distinguishable biomarkers which could be detected for months after dosing. The availability of multiple biomarkers for simultaneous use

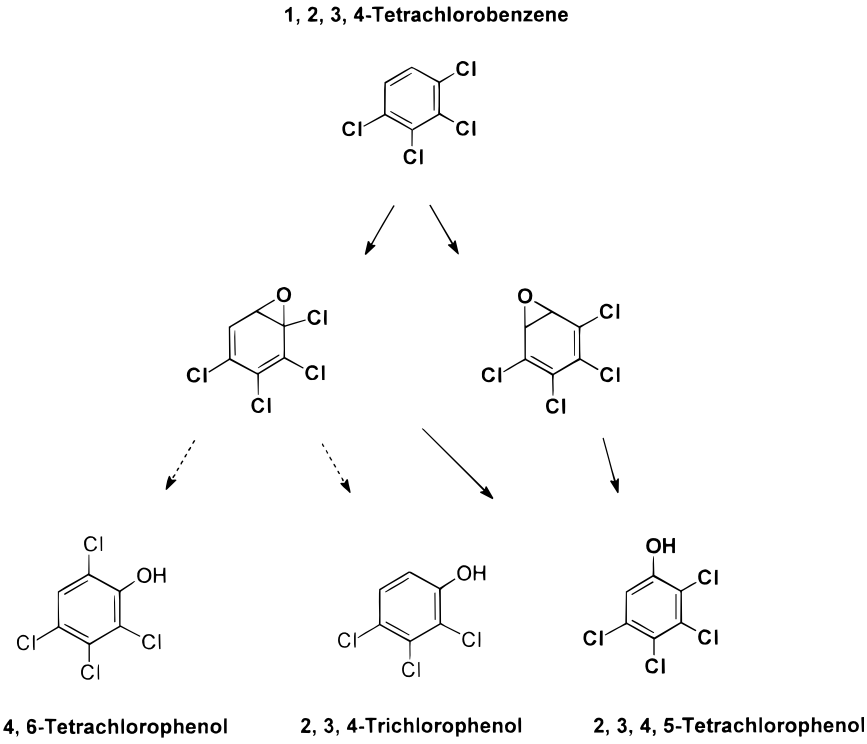

Fig. 6. Proposed scheme for the metabolism of 1,2,3,4-TeCB by coyotes.

on animals of different species, age, sex, location, etc. increases the potential complexity and knowledge to be gained from biomarker studies of predator behavior.

The data comparing feces residues versus different PeCB dosage levels (Fig. 2) show that the different dose levels used in this study resulted in similar feces residue levels. As there was a proportional relationship between oral dose and adipose tissue residues, it appears that solubility in serum is a limiting factor to obtaining high feces residue levels. This is supported by the data in Fig. 3; as adipose tissue levels increased, the size of the slope of the adipose tissue versus feces residue levels decreased.

2,3,4,5-TeCP was detected as a fecal metabolite in PeCB-dosed coyotes. The mammalian metabolism of aromatic compounds to phenols is generally a detoxification reaction for a wide variety of exogenous substrates. Numerous studies have revealed that these biotransformations are mediated *via* hepatic monooxygenases through intermediate arene oxides.[16] The formation of 2,3,4,5-TeCP from 1,2,3,4-TeCB and PeCB probably proceeds through an arene oxide intermediate as depicted in Figs 6 and 7. For 1,2,3,4-TeCB, the rearrangement of the intermediate to 2,3,4,5-TeCP is driven by the low propensity for breaking the C–Cl bond. The C–Cl bond remains intact in the formation of 2,3,4,5-TeCP from either of the postulated 1,2,3,4-TeCB arene oxide intermediates. Theoretical metabolites 2,3,4-trichlorophenol and 2,3,4,6-TeCP were not detected in this study. The formation of 2,3,4-trichlorophenol would require cleavage of the C–Cl bond and subsequent loss of Cl. The formation of 2,3,4,6-TeCP would require a rearrangement of the arene oxide involving a NIH shift of the Cl. This observed stability of the C–Cl bond is consistent with reports postulating arene oxide intermediates for the formation of phenolic metabolites

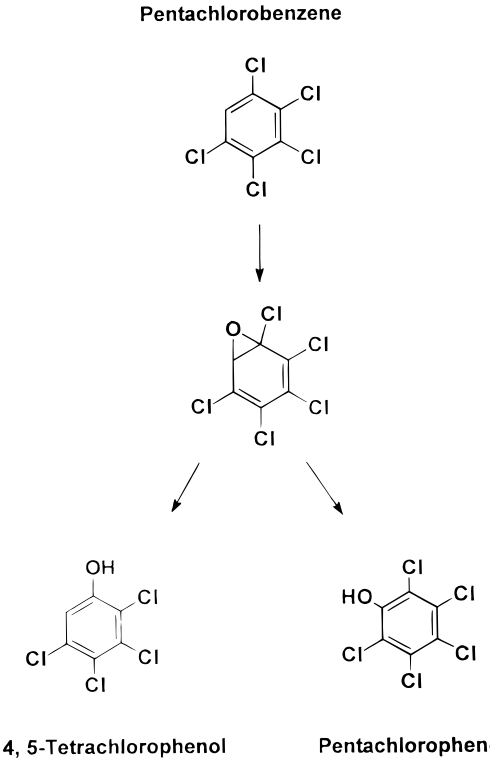

Fig. 7. Proposed scheme for the metabolism of PeCB by coyotes.

from tri- and tetra-chlorinated benzenes.[16,17] For the metabolism of 1,2,3-trichlorobenzene *via* a proposed arene oxide intermediate, Kohli *et al.*[17] reported a 11 : 1 ratio for the formation of 2,3,4-trichlorophenol (which requires no C–Cl or NIH shift cleavage): 2,3,6-trichlorophenol (which requires a NIH shift rearrangement). 2,3,4,5-TeCP also has been reported as a metabolite of 1,2,3,4-TeCB and PeCB in rabbits.[17] This study reported that 20% of 1,2,3,4-TeCB was converted to 2,3,4,5-TeCP whereas only 1% of PeCB was converted to 2,3,4,5-TeCP. Also consistent with our results, Kohli *et al.* did not detect any 2,3,4,6-TeCP in rabbits dosed wit 1,2,4,5-TeCB. Rozman *et al.*[8] reported 2,3,4,5-TeCP and PeCP as metabolites of PeCB in Rhesus Monkeys. Engst *et al.*[18] reported 2,3,4,5-TeCP and PeCP as fecal metabolites of PeCB in rats.

The metabolism of 1,2,3,4-TeCB may account for its rapid elimination as compared to 1,2,4,5-TeCB and PeCB. It has been reported that nearly half the dose of 1,2,3,4-TeCB was oxidized to tetrachlorophenols in rabbits, whereas for 1,2,4,5-TeCB, metabolism accounted for less than 5% of the dose.[19] Chu *et al.*[20] reported higher adipose residues for rats dosed with 1,2,4,5-TeCB as compared to rats dosed with 1,2,3,4-TeCB. The increased metabolism of the latter as compared to the former and PeCB was likely due to the fact that the two vicinal unsubstituted positions on 1,2,3,4-TeCB were more easily oxidized than were aromatic systems which contained no vicinal unsubstituted positions, as found in 1,2,4,5-TeCB and PeCB. This trend has also been noted for the metabolism of trichlorobenzenes where vicinal unsubstituted 1,2,4- and 1,2,5-trichlorobenzenes were hydroxylated to a much greater extent than was 1,3,5-trichlorobenzene which contains no vicinal unsubstituted positions.[21]

In this study, metabolites of PeCB were detected in all coyotes receiving doses of 520 mg while 1,2,3,4-TeCB metabolites were detected in all coyotes dosed with 100 mg. As compared to 1,2,3,4-TeCB, the lack of vicinal unsubstituted ring positions in PeCB probably accounts for its lower rate of metabolism (and increased persistence). PeCP and lesser quantities of 2,3,4,5-TeCP were detected as fecal metabolites of PeCB-dosed coyotes. Both of these metabolites could be formed *via* the arene oxide intermediate proposed in Fig. 7. Of the two metabolites, PeCP was more prevalent than 2,3,4,5-TeCP. 2,3,4,5-TeCP is probably formed *via* the less-favored metabolic route as it requires cleavage of the C–Cl bond and subsequent loss of Cl. No C–Cl bond cleavage is required for the formation of PeCP.

The results of this study indicate that 1,2,4,5-TeCB and PeCB have potential as physiologic biomarkers for wildlife studies. However, in field situations, potential interferences from other sources of PeCB or TeCB could affect the utility of this biomarker technique. For example, PeCB is often present as a low-level impurity ($\leq 0{\cdot}1\%$) in formulations of the fungicide pentachloronitrobenzene (PCNB).[22–25] In dogs fed a 5 mg kg^{-1} PCNB diet for two years, PeCB was detected in feces at 7 $\mu g\ kg^{-1}$. Neither TeCB nor PeCP was detected in feces.[24,25] In wildlife situations, however, it is unlikely that potential exposure of coyotes to PCNB would result in fecal PeCB levels which would significantly affect the quantification of PeCB in feces and hence its use as a biomarker.

When dissolved in mineral oil, 1,2,4,5-TeCB and PeCB can be administered to coyotes orally, as is the practice for most field applications of chemicals or by IM injection, which is practical for use with captured wildlife.[26,27] Though adipose tissue contained the highest concentrations of the biomarkers and potentially offers longer detectability than feces, its field sampling applications are limited, mostly restricted to post-mortem sampling. Feces are the most practical and cost-effective matrix for field sampling. They are easily collected and fecal residues are indicative of total body burden, as feces residues correlated well with adipose tissue levels. Fecal samples yielded detectable marker residues for up to six months post-dosing.

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Mention of companies or commercial products does not imply recommendation or endorsement by USDA. Product names are mentioned solely to report factually on data and to provide specific information.

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